REMARKS

Claims 1-9, 11, 13 and 22-32 were pending in the case at the time of the Final Office Action. Claims 1-9, 11, 13 and 22-32 were rejected. Thus, claims 1-9, 11, 13 and 22-32 are currently under consideration.

EXAMINER INTERVIEW SUMMARY

On Thursday, May 28 2009, Attorney Fairman conducted a telephonic interview with Examiners Shahnan-Shah and Mondesi. The subject of the interview was the art cited under § 102 as anticipating the instant invention. Attorney Fairman explained that Nakamori could not anticipate the instant invention because Nakamori lacked required elements of the claims. Specifically, the instant claims require a) directed genetic modification resulting in b) a modified microorganism that results in c) an inability to grow followed by generations of growth under selective pressure that yields d) an evolved microorganism that is capable of growth on the defined medium that does not support the modified microorganism. In contrast, Nakamori teaches only the random genetic mutagenesis using *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine to select, on complete medium n-methionine overexpressors. Attorney Fairman noted that there simply is no comparison between the two and those of skill in the art would, in fact, find it preposterous that an individual would equate random mutatgenesis with a directed genetic modification.

With regard to WO 93/177112 (Lievense), Attorney Fairman also articulated that the requirements of the present claim are missing from Lievense. Specifically, Lievense teaches the insertion of foreign genes into a microorganism such that the modified microorganism can produce methionine. As with Nakamori, the microorganism is cultured on complete medium and in no way is its growth inhibited by the genetic modification, as is required by the instant claims. Therefore, Lievense is also incorrectly applied to the instant invention.

The Examiners' requested that Attorney Fairman provide a more complete description of the process of directed modification and where such modifications were articulated in the specification. In response, Attorney Fairman has provided explicit citations to the definitions (already provided in previous responses) as well as examples of the making such modifications, which in contrast to Nakamori require knowing the nucleotide sequence of a target gene. In addition, in this response Attorney Fairman provides a scholarly article (Boyartchuk, V. and Dietrich, W., discussed below) that summarizes some methods of directed genetic modification,

identifying a) that "directed genetic modification" is a term of art recognized by those of skill in the art; and b) the term "directed genetic modification" cannot be equated with random mutagenesis.

Double Patenting Rejection

Claims 1, 2, 3, 8, 9, 11, 13, 14 and 22-32 were provisionally rejected on the grounds of non-statutory obviousness-type double patenting as being unpatentable over claims 13-14 and 38-49 of copending Application No. 10/546,139.

This rejection is overcome, at least, for the following reasons.

A terminal disclaimer over U.S. Patent Application No. 10/546,139 is provided herewith.

Rejection under 35 U.S.C. § 102

Claims 1-4 and 8, 9, 11 and 13-14 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Nakamori et al. (Applied Microbial Biotechnology, vol. 52, pp. 179-185, 1999) (hereinafter "Nakamori").

This rejection is overcome, at least, for the following reasons.

Nakamori Does Not Teach Directed Mutagenesis Followed By Evolution

Claim 1 and claims 2-9, 11, 13-14, and 22-32 depending therefrom, require the directed mutation of a gene of interest (step a) followed by growing the microorganism over a period of many generations (several days) in a defined, minimal media in order to develop new (compensating) pathways to overcome the induced auxotrophy created by the directed modification. See, for example, paragraphs [0097], [0125], [0136], [0199], [0204], [0208] and [0356] of the published application U.S. Publication No. US05/0054060. In contrast, Nakamori discusses the generation of random genetic mutations in E. coli using (*N*-methyl-*N*'-nitro-*N*-nitrosoguanidine). ***Applicants respectfully submit that Directed Genetic Modifications cannot be made randomly using mutagenic chemicals.***

Directed Genetic Modification Is Defined In The Specification

"According to the invention a "modified microorganism' is a microorganism obtained by forming controlled modifications, i.e., that are not the result of the process of evolution. Examples of such a modification are the direct mutation or deletion of a gene or the direct modification of a promoter." U.S. Pub. No. US 2005/0054060 at [0038]. With respect to the foregoing definition, those of skill in the art recognize that a "controlled" modification cannot be made randomly. Further, a "directed mutation" cannot be made without knowing a sequence of a target gene in order to insert or delete a portion of a gene. Similarly a directed modification of a promoter of a gene cannot be made without a predesigned method for its making, i.e., the knowledge of the sequence of the gene and a strategy for making the insertion or deletion that is the nature of the modification. Further, this process is described in the specification. For example, "the inactivation of a gene is carried out preferably by homologous recombination . . . The principal of a protocols is briefly as follows: a linear fragment obtained in vitro is introduce into the cell; this fragment comprises the two regions flanking the gene and at least one gene of selection between these two regions (generally a gene of resistance to an antibiotic); the fragment therefore presents an inactivate gene . . . Id. at [0055]. Thus as defined in the specification, and as described the process of making a modified microorganism by a genetic modification requires a knowledge of the sequence of a target gene and the sequence used in modifying the target or, the target is modified by a modification of its control sequence (e.g., promoter, repressor etc.) Applicants however, submit that such a rational modification is not and cannot be made by a random mutation because the target is not known. Further, Applicants respectfully submit that while a random mutation may eventually identify a genetic source, that cannot happen until millions of randomly generated mutant are selected via some phenotype.

The Process Of Evolving The Microorganism Is Clearly Described In The Specification

As discussed in the specification, the clones containing the original directed genetic mutation are cultured for 6 days. Applicants note that the evolution of the strain takes several generations of micro-organism growth. In this context, it should be appreciated that the life span of a microorganism is quite finite compared with those of multi-cellular organisms. For example, the doubling time of E. coli grown in the laboratory is from 20 to 30 minutes. See for example, "Biology – E. Coli (http://en.allexperts.com/q/Biology-664/E-Coli-2.htm). Therefore, the initial culture and evolution of the modified bacteria discussed in the examples (see, [0227]-

[0246]) comprises 6 days of initial culture in defined medium for six (6) days. Applicants note that 6x24 = 144hours / .5 (30 min) = ***288 generations***. Applicants further submit that the passage of 288 generations provides time for evolution of the modified strain and allows time to identify the flasks enumerated in Table 1 which show an increase in optical density, e.g., modified bacteria that evolve a pathway allowing the use methylmercaptan for the production of methionine. As described in the specification, successive reseedings of cultures from flask 2 allow the identification of a single particular clone (K1a-F) that is autotrophic from methionine using methylmercaptan as a sulfur source.

Next, as described in the specification, the K1a-F clone undergoes 14 further reseedings in flasks. This process, while requiring the further culturing of the respective aliquots from flask 2, provides for the further evolution of the autotrophic clones for another hundreds or thousands of generations over a similar number of days. Following this selection process, a specific clone (K144 in the example described in paragraphs [[0227]-[0246]) can be isolated.

Following the isolation of K144 in liquid culture, putative clones are spread on minimal medium in plates. The plates are put in a jar in an atmosphere which includes methylmercaptan. It is not until 4 days following incubation of the putative K144 strain on ***minimal*** media that clones appear. Again, using a conservative estimate of 30 minutes for the doubling time of E. coli, four days of incubation until the growth of clones autotrophic for methionine, in the presence of methylmercaptan equates into about 192 generations.

Nakamori, In Contrast, Teaches Neither Directed Mutation Nor Evolution

Nakamori, in contrast, uses random mutagenesis to generate hundreds of thousands (at least) of random mutagenic events. Nakamori then eventually identifies some cells in which an autotrophic **overexpressor** phenotype of methionine is identified. Further genetic analysis eventually finds that **the repressor (*metJ*) is derepressed.** (See, **Title**, Abstract, entire paper). As discussed by Nakamori, and the instant specification, at for example, [0214], [0237], methionine and cysteine act in a negative feedback manner on the *metJ* gene to inhibit the production of methionine when intracellular concentration increases above a certain level. When Nakamori mutates (randomly) the *metJ* gene, the result is the increased production of methionine by ***autotrophic*** super-producers on ***complete media*** increasing the production of methionine by wild-type metabolic pathways. No enzymes are change, evolved or deleted. Only the effect of

metJ in repressing the genes has changed. See, pg. 181, col. 2, results and pg. 182, col. 1, especially, Table 2 and pages 183-185. There is ***no substrate or product that is inhibited and no new substrates are usable*** by the bacteria. Further, this is explicitly stated in the Nakamori application (JP 2000157267) as the problem to be solved. "To obtain a new variation type *metJ* gene having a specific amino acid sequence lowered in activity in *repressing* methionine biosynthesis." JP 200015726 (Nakamori, Abstract).

Therefore, Nakamori cannot anticipate the instant invention at least because the instant claims ***require*** "generating a genetic modification in a gene of interest ***wherein the production or consumption of a substrate is inhibited.***" Again, Applicants emphasize that Nakamori does not teach, does not suggest nor motivate anyone to inhibit the consumption or production of a substrate. Nakamori is only interested in using the wild-type metabolic pathways, without the feedback inhibition, due to increased levels of methionine.

Further, as Applicants pointed out to the Office in the reply to the action of July 16, 2007, filed on September 14, 2007, Nakamori cannot teach directed genetic modification. The methods disclosed by Nakamori et al. do not include a ***directed genetic modification*** as is required by instant claim 1. In particular, the Offices' attention is directed to Sugimura et al. (*Nature* 210, 962 - 963 (28 May 1966) and to Shen et al. *J. Proteome Res.,* **5** (2), 385 -395, 2006.

> *N*-Methyl-*N*'-nitro-*N*-nitrosoguanidine (NG) is known to be a potent mutagenic substance for *Escherichia coli* [1], *Salmonella typhimurium* [2], and *Chlamydomonas reinhardi* [3]. Induction of chromosomal aberrations in the cells of root meristems of *Vicia faba* by NG has been reported[4]. It is claimed that NG is the most potent mutagen yet discovered, since NG induced at least one mutation per cell of *Escherichia coli* treated at the optimal condition permitting 50 per cent survival[5]. The production of a number of multi-site mutants resulting from the accumulation of single-site mutations has been noticed. (Sugimura et al., Abstract)

> Alkylating agent MNNG (*N*-methyl-*N*'-nitro-*N*-nitrosoguanidine) can induce DNA damages which can lead to chromosomal aberrations, mutations, and cell death. Previous reports from our laboratory have found that low concentration of MNNG can induce nontargeted mutations (NTM) at undamaged bases in DNA, clustering of epidermal growth factor receptor (EGFR) and interference of EGFR mediated signaling, as well as activation of endoplasmic reticulum stress. Thus, the cellular responses to MNNG

> exposure are very complex, and can be triggered by signals originated from different compartments of the exposed cells. To further probe the molecular mechanisms involved in cellular responses to MNNG treatment, and to find potential biomarkers for MNNG induced stress condition, we performed proteomic analysis of whole cellular proteins from human amnion epithelial cells after exposing to MNNG at 3 different doses. More than 80 proteins were affected by MNNG treatment, and 71 proteins among them were identified using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry. These proteins take part in a wide variety of cellular processes including regulation of transcription, metabolism, cytoskeleton organization, cell cycle, cell proliferation, signal transduction, transportation, etc. The significance of these proteins in the genesis of MNNG induced cellular defensive response and hazardous effect remains to be elucidated, the results may also give a clue for biomarker search for monitoring the exposure risk of MNNG. (Shen et al., Abstract)

With regard to the actions of N-methyl-N'-nitro-N-nitrosoguanidine, Applicants point out that the use of N-methyl-N'-nitro-N-nitrosoguanidine to promote random mutagenic events is incompatible with claim 1 of the present invention which requires directed genetic modification. For this reason, at least, the rejection should be withdrawn.

Therefore, not capable of teaching ***directed*** genetic modification, Nakamori only uses random mutagenesis to identify mutants that are derepressed for the production of methionine when grown on complete media utilizing conventional metabolic pathways. As previously pointed out, only one of the at least hundreds of thousands (and more likely millions) of random mutagenic events generated 5500 were identified as analogue resistant and of these, just seven were halo forming using the *pediococcus acidilactici* methionine assay. Of these seven, only four produced appreciable quantities of methionine. Nakamori Table 1. Further, Nakamori provides a complete medium, excepting methionine, the production of which, by the inoculum, allows the *pediococcus acidilactici* to grow. Thus, the procedure of ***Nakamori is not directed genetic modification and no cells were selected in which the production or consumption of a substrate was inhibited as is required by the instant claims.*** In fact, the opposite selection criteria were used, cells were selected in which the production was de-repressed. For these reasons alone, the rejection is overcome and should be withdrawn. Applicants respectfully request the same.

Further, in light of Nakamori's explicit statement that the mutations introduced therein are random mutations, Applicants request that the Examiner particularly point out where in Nakamori directed genetic modification (or mutation) is taught. Further, while the Examiner states on page 6 of the office action that the bacteria treated with N-methyl-N'-nitro-N-nitrosoguanidine exhibit a "specific mutation in a metabolic pathway" it is not a directed mutation but a selection from random mutagenesis.

In addition, applicants respectfully submit that the Examiner misunderstands the meaning of mutation. Therefore, for the Examiner's benefit the following discussion is provided.

> The mechanisms of evolution—like natural selection and genetic drift—work with the *random* variation generated by mutation.
>
> Factors in the environment are thought to influence the rate of mutation but are not generally thought to influence the direction of mutation. For example, exposure to harmful chemicals may increase the mutation rate, but will not cause more mutations that make the organism resistant to those chemicals. In this respect, mutations are random—whether a particular mutation happens or not is generally unrelated to how useful that mutation would be.
>
> Evolution 101

http://evolution.berkeley.edu/evosite/evo101/IIIC1aRandom.shtml

In addition, Applicants note that the term "directed genetic modification" is art recognized. Specifically, the Office is referred to Genes Immun. 2002 May;3(3):119-22.

> In brief, there are a few mutually complementing genetic approaches that are used to study the host immune response. These approaches can be roughly split into two classes. Many investigators analyze alterations in the host response that are induced by directed genetic modification of an organism. Typical genetic modifications include mouse gene knock-outs, knock-ins, and introduction of transgenes.

Boyartchuk V, Dietrich W., Genetic dissection of host immune response, Genes Immun. 2002 May;3(3):119-22. (Copy provided herewith for the Examiner's convenience.)

Thus, in light of the above discussion, Applicants submit that the Office is mistaken and that Nakamori cannot teach a process of "Directed Genetic Modification" when Nakamori does not and, in fact Nakamori identifies that the process used is random

mutagenesis. Therefore, for this reason alone, the rejection is overcome and should be withdrawn. Applicants respectfully request same.

For the Examiner's convenience, the Applicants are providing a table of some of the most salient differences between Nakamori and the instant invention.

	The present invention	**Nakamori et al** (see page 180, "Materials and methods" section)
Step 1	Directed genetic modification of a microorganism, in particular attenuation of a specific gene by homologous recombination, for suppressing an enzymatic activity (see paragraphs 74, 75, 76, 77).	Random mutagenesis of E coli cells by treatment with N-methyl-N'-nitro-N-nitrosoguanidine, a mutagenic agent inducing random mutations;
Step 2	Genetically modified microorganism are cultivated for numerous cycles (about six days) in a flask, in a medium comprising methylmercaptan or sodium methylmercaptide, or sulphur compound as co-substrate (see [75]) and free of the metabolite, the production of which is inhibited by the previously done modification [21]. This is a step of evolution such as defined in the specification: See in particular paragraphs [3], [78], [199], [204], and [208].	No corresponding step. Mutagenized cells are spread onto plates containing various quantities of L-methionine analogs and incubated for 72h; this is performed in the aim to select cells that have acquired a mutation in the methionine biosynthesis pathway; this is not an evolution step
Step 3	Selection of microorganisms able to grow on said defined medium.	Resulting colonies (i.e. colonies resistant to the methionine analogs) are collected and tested for L-methionine production.

Concerning the first step:

It is clear for the man skilled in the art that there is a difference between performing a mutagenic treatment that induces random mutations in strains, and performing a homologous recombination that is directed in order to inactivate or delete a gene, in the aim to suppress a specific enzymatic activity.

The examiner states that *"Nakamori et al. teach preparing a modified microorganism with genetic modification of cells of an initial microorganism so as to inhibit the production or consumption of a metabolite (methionine) ...."*

This is not correct: Nakamori et al teach a random mutagenesis of cells, which is not directed to the inhibition of production or consumption of a metabolite; cells having acquired a modification in the metabolite production/ consumption pathway will be selected long after this step.

Moreover, the examiner states that *"Nakamori et al teach directed genetic modifications"* (see page 6 of the Official notice) which is not true: the man skilled in the art understands, from Nakamori's teaching, that the mutations are randomly generated.

Concerning the second step:

Both in their goal and in the techniques used for performing them, "growth of the microorganisms" is different. Culture on plates for 72 hours, and culture in flasks for 6 days (the present invention), are different processes.

The examiner states that *"Nakamori et al. teach culturing the modified microorganism thereby obtained on said defined medium to cause it to evolve, where the defined medium can contain a co-substrate to allow such evolution ..."*

This is not correct: Nakamori et al teach a culture on plates for 72 hours. This is not an evolution step, but simple selection of mutants generated through the preceding mutagenesis step. (Nakamori et al., 1999) It corresponds to the step 3 of the present invention.

As previously discussed, the process can be schematically represented as:

The Instant Process:

1 – Directed genetic modification of a microorganism;

2 – Culturing only those micro organisms in which the directed genetic modification was made. Specifically, on a minimal media devised for the selection and evolutionary pressure of the desired biosynthetic pathway;

3 – Obtaining those microorganisms that grow in step 2.

Schematically, the instant methods can be represented as:

$$\mathbf{I_o \rightarrow M_o \rightarrow E_o}$$

Where:

$\mathbf{I_0}$ is the initial microorganism;

$\mathbf{M_0}$ is the modified microorganism; and

$\mathbf{E_0}$ is the evolved microorganism.

Nakamori:

1 – Random mutagenesis of bacteria

2 – Isolation of autotrophic superproducers on complete medium.

Schematically, this process can be represented as:

$$\mathbf{I_0 \rightarrow R_0}.$$

Where:

$\mathbf{I_0}$ is the initial microorganism;

$\mathbf{R_0}$ is the randomly mutagenized microorganism.

Therefore, as discussed above, Nakamori cannot anticipate the instant invention, at least because the instant invention requires that the initial organism be modified by (a) a directed genetic modification such that (b) the production or consumption of a substrate is inhibited. Nakamori teaches neither of these elements. For at least these reasons, the rejection of claim 1 and all claims depending therefrom over Nakamori is overcome and should be withdrawn. Applicants respectfully request same.

Claims 1-7 stand rejected under 35 U.S.C. § 102(b) as being anticipated by WO 93/177112 (Lievense).

This rejection is overcome, at least, for the following reasons.

Lievense Does Not Teach Preparing a Modified Microorganism that is Impaired in Its Ability To Grow

Claim 1 *requires* at least the elements (a) preparation of a modified microorganism by directed genetic modification, impairing the ability of the microorganism to grow on a defined medium; (b) culturing the modified microorganism; and (c) selecting a modified microorganism whereby an evolved microorganism is prepared.

In contrast, Lievense merely inserts foreign genes in bacteria allowing the bacteria to use non-native substrates to produce methionine. ***The growth of the transformed bacteria taught by Lievense is not impaired, as is required by the claims.***

Lievense Does Not Teach Evolved Bacteria.

Because the growth of the bacteria modified by Lievense is not impaired, there is no selective pressure to "evolve" the bacteria. This process is discussed in the specification at, for example, paragraphs [0002], [0017], [0023], [0024] and [0078] reproduced below.

> [0002] The preparation of microorganisms with modified properties is a widely used process. The aim is either to cause the microorganisms to evolve by letting them grow on a growth medium with a factor that exerts a selection pressure, so as to select those microorganisms able to resist that pressure, or to introduce one or more heterologous genes by means of widely used genetic engineering methods, in order to lend the microorganisms new phenotypic features associated with the expression of said heterologous gene or genes. This evolution can be favored by the use of mutagenic agents well known to those skilled in the art.
>
> [0023] According to the invention "selection" is a culture method used to select microorganisms that have evolved in such a way that a modification does not affect growth. A preferred application is a continuous culture method, carried out by applying increasing rates of dilution so as to conserve in the culture medium only those microorganisms with a growth rate equal to or greater than the imposed rate of dilution.
>
> [0024] According to the invention an "evolved gene" is a sequence of nucleic acids (comprising A, T, G or C) bounded by a stop codon (TAA, TAG, TGA) in phase and possessing, after selection, at least one nucleic acid that is different from the initial sequence, so that the protein coded by that evolved gene differs in at least one amino acid from the protein coded by the initial gene.
>
> [0017] According to the invention an "evolved microorganism" is defined as a microorganism obtained by selection of a modified microorganism. The evolved

microorganism displays at least one difference from the modified microorganism. This difference may, for example, be the improvement of an enzymatic characteristic, or the creation of a new metabolic pathway.

[0078] The procedure for preparing strains according to the invention consists in obtaining, from an initial bacterial strain, a genetically modified bacterial strain presenting at least one modification in a gene coding for an enzyme with a "methionine synthase" (e.g. MetE) or "homocysteine synthase" (e.g. MetC) activity, by a process comprising a step in which the initial bacterial strain is subjected to selection pressure in the presence of the sulfur source specified above, in order to cause the evolution of at least one gene (e.g., metB) in that bacterial strain, so as to restore a "methionine synthase" or "homocysteine synthase" activity in the evolved strain, this restoration of activity not being due to a reversal of the modification made.

In the instant case, **the evolved microorganism is required to grow on the defined medium which the modified microorganism cannot**. Applicants respectfully request that the Office identify what the difference is between the end-product of Lievense genetic manipulation and what then, is the evolved microorganism that the Office identifies. Because, Lievense does not provide a step of evolution, there is no evolved microorganism and the only microorganism other than that of the starting "wild-type" organism is the transformed or "modified" microorganism. Therefore, for this reason alone, the rejection is overcome and should be withdrawn.

In contrast, the process of Lievense does not require evolution because **no gene is impaired, as is required by the claims**, therefore there is no selective pressure and no evolution occurs. In fact, Lievense does not teach the growth of the transformed bacteria on a defined medium, as is also required by the claims. As defined in the specification and cited above:

Further, there is no selective pressure in Lievense because the transformed bacteria are grown in a rich culture medium. In contrast, the instant claims require a defined culture medium. As previously pointed out to the Office, Lievense uses a "soy hydrolysate". Soy hydrolysate by definition cannot be a "defined" medium. Specifically: Example 1 of Lievense describing "the

parent and transformed microbes are cultivated individually in a fermentation medium containing glucose, soy hydrolysate, and inorganic nutrients. The medium is supplemented either with sulfate or sulfide as a source of sulfur for methionine production." WO93/17112 at 7. Applicants point to one description of soy hydrolysate from Millipore, Inc.:

> **Description:** LucraTone Soy F hydrolysate is obtained from the enzymatic hydrolysis (non-animal) of defatted soybean plant flour from Identity-Preserved (IP) sources as it pertains to GMOs. It is fortified with essential amino acids and vitamins.
> **Certification:** Animal-free, GMO free
> **Applications:** LucraTone Soy F supplement is a highly productive hydrolysate with a high carbohydrate content and large quantities of smaller molecular weight peptides. It is easily assimilated by many microorganisms and is a good source of nitrogen for cell culture medium or fermentation substrates.

(http://www.millipore.com/publications.nsf/docs/tb1036en00)

Applicants therefore submit that any medium containing soy hydrolysate is not a defined medium as required by claim 1. Thus, for this reason, Lievense cannot anticipate the present invention and the rejection is overcome.

Lievense Does Not Include The Steps Required By The Claims

For the Examiner's convenience Applicants provide herewith a table identifying some of the most salient differences between Lievense and the instant invention.

Lievense describes a method for enhancing methionine production in a fermentation process, by transforming a microorganism with a homoserine-activating enzyme gene and a sulphur-incorporating enzyme gene.

The aim of these genetic modifications is to improve the production of methionine by over-expressing genes encoding for enzymes involved in the biosynthesis pathway.

This document does not teach any process of evolution, but a process of directed rational genetic modifications.

The process such as taught in WO 93/17112 can be summarized as follows:

	The present invention	**Lievense** (see claim 1)
Step 1	Directed genetic modification of a microorganism, in particular attenuation of a specific gene by homologous recombination, for suppressing an enzymatic activity (see paragraphs 74, 75, 76, 77).	Directed genetic modification of cells with over-expression of a homoserine-activating enzyme gene fragment and a sulfur-incorporating enzyme gene fragment;
Step 2	Genetically modified microorganism are cultivated for numerous cycles (about six days) in a flask, in a medium comprising methylmercaptan or sodium methylmercaptide, or sulphur compound as co-substrate (see [75]) and free of the metabolite, the production of which is inhibited by the previously done modification [211. This is a step of evolution such as defined in the specification: See in particular paragraphs [3], [78], [199], [204], [208].	No corresponding step. Cells are grown in a ***rich*** medium, the goal to let the transformation occurs ; this is not an evolutionary step
Step 3	Selection of microorganisms able to grow on said defined medium.	Resulting cells are collected and tested for L-methionine production

The examiner states that *"WO 93/17112 teaches culturing the modified microorganism thereby obtained on said medium to cause it to evolve..."*. This is incorrect for the following reasons:

Neither the terms "evolution" or "evolve" are used in the text of this patent application;- the "growth period" of the cells is performed only to allow the transformation to occur in cells; see example 1.

Conditions of this growth period are not detailed at all in WO 93/17112, because it is not an important step of the process. It is clear from this document that the capability of the strain to produce methionine comes from the overexpression of heterologous genes, involved in methionine production, and not from any evolution step (non existent in this description).

The process of Lievense can be distinguished from the instant invention because *Lievense does not involve any step of evolution.* The transformed cells of Lievense are not subjected to any "growing" period or allowed to "evolve" any compensatory pathway but, rather they are

transformed with known genes to produce desired products using a well understood pathway that has been characterized (thus, the ability to transform with the desired genes) and grown in a complex medium simply for the purpose of producing L-methionine. In Lievense, there is no selective pressure and there is no directed genetic modification which results in the "inhibition of the production or consumption of a substrate." This is an element *required* of the claims. The Office is not free to read into Lievense the instant limitations. The microorganisms of Lievense are not grown in a defined medium and allowed to evolve new pathways to compensate for the autotrophic production of L-methionine. As autotrophs, there is no pressure to evolve any new pathways. These are very different conditions from the present invention.

The process of Lievense can be summarized schematically as:

$$\mathbf{I_o \rightarrow M_o}$$

Where

$\mathbf{I_o}$ is the initial microorganism;

$\mathbf{M_o}$ is the modified microorganism.

Again, the instant method requires:

$$\mathbf{I_o \rightarrow M_o \rightarrow E_o}$$

There are is no step of debilitating genes or biosynthetic pathways in the method of Lievense. Specifically, as stated by Lievense, "*E. coli*, *C. glutamicum*, and *B. flavum* are de-regulated for homoserine overproduction by classical or genetic engineering methods. The sulfhydrylation route to methionine is introduced into these microbes by transforming them with plasmids encoding homoserine acetyltransferase, o-acetylhomoserine (thiol)-lyase, and homocysteine methylase. The parent and transformed microbes are cultivated individually in a fermentation medium containing glucose, soy hydrolysate, and inorganic nutrients." Lievense at 7, Example 1. In contrast to the present invention, where a native enzyme is debilitated resulting in a modified organism that is auxotrophy, and which must then develop pathway to survive auxotrophy resulting in autotrophy, Lievense merely take an autotrophic organism and increases its autotrophic production.

Lievense does not teach or even contemplate "the genetic modification of cells of an initial microorganism, so as to *inhibit* the production or consumption of a metabolite." This is a step that is **required** by instant claim 1. Lievense merely teaches the derepression of cells followed by the overproduction of the methionine biosynthetic pathway wherein the same pathways and same substrates are used. Further, Lievense does not teach the attenuation of any gene involved in the production or consumption of a metabolite, *Lievense merely teaches the overexpression of a known heterologous gene*. This process is not at all analogous to the evolved expression of endogenous genes. Therefore, for these reasons alone, the rejection over Lievense is overcome and should be withdrawn. Applicants respectfully request same.

Claims 22-32 were rejected under 35 U.S.C. § 102(b) as being anticipated by Nakamori.

This rejection is overcome, at least, for the following reasons.

Claims 22-32 depend from claim 1, Nakamori, not teaching the elements of claim 1 cannot make obvious claims dependent therefrom. The rejection of dependent claims 22-32 being therefore overcome withdrawal of the rejection is respectfully requested.

CONCLUSION

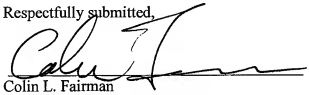
In view of the foregoing, it is respectfully submitted that each of the pending claims is in condition for allowance, and a Notice of Allowance is earnestly solicited.

If the Commissioner determines that any additional fee under 37 C.F.R. §§ 1.16 to 1.21 is required for any reason, the Commissioner is hereby authorized to deduct said fee from Fulbright & Jaworski L.L.P. Account No.: 50-1212/CABR-029/US.

The Examiner is invited to contact the undersigned attorney at (612) 321-2237 with any questions, comments or suggestions relating to the referenced patent application.

CUSTOMER NUMBER **38824**

Respectfully submitted,



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